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Source: Journal of Economic Entomology, 113(1) : 108-114

Published By: Entomological Society of America

URL: https://doi.org/10.1093/jee/toz283

Journal of Economic Entomology, 113(1), 2020, 108–114
doi: 10.1093/jee/toz283
Advance Access Publication Date: 1 November 2019
Research

Biological and Microbial Control

Impact of Imidacloprid Soil Drenching on Survival, Longevity, and Reproduction of the Zoophytophagous Predator *Podisus maculiventris* (Hemiptera: Pentatomidae: Asopinae)

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Subject Editor: Eric Wajnberg

Received 24 July 2019; Editorial decision 25 September 2019

Abstract

Systemic insecticides when applied as seed treatments or soil drenches are often more toxicologically selective for natural enemies than target pests. This may not be the case, however, for omnivorous predators, which are at risk of extended exposure to systemically applied pesticides through ingestion while feeding on treated plants for nutrients or water. Such exposure may kill or have sublethal consequences for these natural enemies, compromising their role as biocontrol agents of agricultural pest species. The spined soldier bug, *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae: Asopinae), is an important zoophytophagous biocontrol agent (i.e., able to substitute zoophagy by phytophagy for survival) that may be exposed to systemic insecticides in many agricultural systems. We, therefore, examined effects on *P. maculiventris* following exposure to cabbage plants subject to soil-drench treatments with imidacloprid, a systemic neonicotinoid insecticide. Predator survival, development, body weight, and reproduction were recorded. Imidacloprid significantly affected nymph survival and adult emergence, but not duration of the nymphal period or adult body weight. At one-twentieth the recommended field rate for whitefly and aphid management, imidacloprid treatments reduced longevity, fecundity, and fertility of female predators. These findings demonstrate that soil treatments with systemic insecticide can negatively impact zoophytophagous natural enemies.

Key words: systemic insecticide, spined soldier bug, sublethal effect, neonicotinoid

Soil or seed treatment applications of systemic pesticides is an important strategy to prevent damage caused by early season agricultural pests (Jeschke et al. 2011, Simon-Delso et al. 2015). Neonicotinoids insecticides such as the imidacloprid are commonly applied in this way to suppress populations of a wide variety of insects, including piercing-sucking pests like whiteflies, aphids, and leafhoppers (Nauen et al. 1998, 1999; Wilde et al. 2004; Tomizawa and Casida 2005; Elbert et al. 2008). An advantage of systemic insecticide applications encouraging their use over foliar sprays is that such applications tend to reduce exposure to nontarget organisms, with exposure being limited mainly to herbivores that feed on plant tissues or materials (e.g., pollen or nectar) (Ruberson et al. 1998, Cloyd and Bethke 2011, He et al. 2012, Main et al. 2018). However, this alleged safety to nontarget organisms, or ecological selectivity, has been recently called into question (Prabhaker et al. 2011, Gontijo et al. 2018).

The uptake of systemic pesticides by seeds or plant roots allows them to remain within the plant for several weeks (Juraske et al. 2009, Akoijam and Singh 2014, Sharma and Singh 2014). This may extend pesticide exposure of nontargeted organisms that ingest foliage, pollen, nectar, or plant sap (Cloyd and Bethke 2011, Simon-Delso et al. 2015). Such exposure route has been well recognized for pollinators (Blacquière et al. 2012, Van der Sluijs et al. 2013, Barbosa et al. 2015, van Lexmond et al. 2015). Though they have received less attention than pollinators, omnivorous natural enemies are also potentially exposed to systemic pesticides when feeding on contaminated plant tissues (Moser and Obrycki 2009; Torres et al. 2010; Gontijo et al. 2014, 2015; Moscardini et al. 2015).

The spined soldier bug *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae: Asopinae) is an important zoophytophagous predator (De Cock et al. 1996, Mohaghegh et al. 2001, De Clercq et al. 2003, Montemayor and Cave 2011, Koch et al. 2017). This insect occurs in a wide range of agricultural systems in North America (Herrick and Reitz 2004, De Clercq 2008), and was recently detected in South America as well (Silva et al. 2018). Easily reared, the spined soldier bug is commonly produced and sold by biological control companies. Although it is an important predator of agricultural pests (McPherson 1980, De Cock et al. 1996, Mohaghegh et al. 2001, De Clercq et al. 2002, Koch et al. 2017), *P. maculiventris* is omnivore and regularly feeds on plant xylem to complement its diet and water requirements (Ruberson et al. 1986). This omnivory may increase exposure of *P. maculiventris* to systemic pesticides or their metabolites.

Previous studies reported high acute mortality of imidacloprid on *P. maculiventris* (De Cock et al. 1996, Cutler et al. 2006), and some negative effects of systemic pesticides on other zoophytophagous predators (Torres et al. 2003; Rogers et al. 2007; Moser and Obrycki 2009; Gontijo et al. 2014, 2015, 2018), but little information is available on the effects of insecticide soil drenching on plant-feeding (zoophytophagous) predators. Resende-Silva et al. (2019) reported negative effects of imidacloprid soil drenching on the predatory activity of *P. maculiventris* after short-term exposure. However, plant-feeding predators like *P. maculiventris* can be continuously exposed to pesticides when applied as soil drenches, and the ramifications of such exposure on sublethal biological endpoints is unclear.

In the current study, we investigated whether exposure of *P. maculiventris* to imidacloprid via phytophagy on soil-drenched treated plants would compromise the predator's survival, developmental, body weight, longevity, and reproduction. We used four imidacloprid concentrations, rearing of *P. maculiventris* on treated cabbage plants, and insecticide-free *Tenebrio molitor* (Linnaeus) (Coleoptera: Tenebrionidae) pupae as prey. We expected significant negative effects of the insecticide on the predator because of previous negative effects reported for imidacloprid foliar sprays (De Cock et al. 1996, Cutler et al. 2006), high toxicity of imidacloprid on piercing-sucking pest species (Tomizawa and Casida 2005, Casida and Durkin 2013), and reported negative effects on other nontarget species (Torres et al. 2003; Rogers et al. 2007; Moser and Obrycki 2009; Gontijo et al. 2014, 2015, 2018; Moscardini et al. 2014).

Material and Methods

Insects and Plants

Podisus maculiventris were a colony maintained at the Faculty of Agriculture, Dalhousie University (Nova Scotia, Canada), originally established in 2016 from adults and larvae purchased from the Bug-Factory (Nanoose Bay, British Columbia, Canada). *Tenebrio molitor* (Linnaeus) (Coleoptera: Tenebrionidae) were purchased as needed from a local pet store (Pet Valu, Truro, Nova Scotia, Canada), and maintained following methods by de Castro et al. (2013). The predators were maintained in an insecticide-free environment with mealworm larvae as prey, following Cutler et al. (2006), but using cabbage leaves as the only water source.

Cabbage seeds (*Brassica oleracea* capitata group, cv. Golden Acre) were sown in 48-cell plastic trays filled with commercial organic substrate (Pro-mix, Rivière-du-Loup, Québec, Canada), and kept in greenhouse under ambient temperature (20–25°C) and free of pesticides. Twenty-five to 30 d after the emergence, same-size seedlings (4–6 expanded leaves) were individually transplanted into plastic pots (14 cm × 10.5 cm [diameter by height]), and filled with Pro-mix substrate. Plants were watered immediately after the transplantation and thereafter watered as needed. Twenty-four hours after the transplanting, plants were subjected to soil-drenching treatments.

Soil-Drenching Treatment

Imidacloprid (Admire 240, Flowable Systemic Insecticide, Suspension, Bayer Crop Science, AB, Canada) was used at 240 g of active ingredient (a.i.) per hectare (ha), which is the label rate recommended against whiteflies (*Bemisia tabaci*) in brassica crops in Brazil (MAPA 2018), and against cabbage aphids in Canada (Bayer Crop Science Canada 2018). The imidacloprid treatment concentrations used were calculated based on the recommended label rate and a cabbage spacing of 80 × 60 cm (20,833 plants per ha). Treatments of 0.50, 1.01, 5.04, or 10.08 mg a.i. per pot were used, corresponding to one-twentieth, one-tenth, half, and full field recommended rates, respectively.

Imidacloprid was diluted in water, and 15 ml of solution was applied to the substrate at the base of each cabbage plant following the recommendation for drench application (MAPA 2018). Water alone was applied to control plants. After soil-drench applications, plants were maintained for 1 wk in a greenhouse and watered regularly, without exceeding the water holding capacity of the substrate, and then transferred to a walk-in environmental chamber maintained under conditions of 22 ± 2°C, 65 ± 5% RH, and 16:8 (L:D) h photoperiod. The experiments with insects described below were carried out in this environmental chamber.

Nymph Survival and Development

Effects of imidacloprid soil drenches on survival and development of *P. maculiventris* were assessed by rearing third-instar nymphs on treated cabbage plants until adult emergence. This experiment was conducted using a randomized complete block design with eight blocks and one plant per treatment for each block. Plants from the same transplant date and dilution series constituted a block.

A 3–4 cm layer of sand was added to the surface of each pot before transferring nymphs to prevent contact of insects with the contaminated substrate (Uhl et al. 2015). Subsequently, eight newly molted third-instar nymphs (<24 h old) were randomly selected from a mass reared colony and carefully transferred to each plant using a small soft-bristle brush. Across eight experimental blocks, this resulted in a total of 64 insects per treatment. Mealworm pupae of similar sizes without previous exposure to insecticide were placed on the soil surface as prey, and were prevented from escaping using methods described by Torres et al. (2003). Thus, treated plants were the only source of insecticide exposure for the predator. For each potted plant, mealworms were provided to *P. maculiventris* nymphs at a ratio of 1:1 per day. This rate of prey prevented cannibalism. Although *P. maculiventris* would move down to the soil surface to feed on the mealworm pupae, most of the time the predator remained on plants. To prevent insect escape and enable air exchange, each potted plant was covered with a micro-perforated plastic bag (4–5 holes per cm^2, PrismPak Inc., Berwick, PA) secured around the pot with a rubber band.

Nymph survival and stage of development were recorded daily. After the daily watering of plants, mealworm cadavers were removed and replaced with healthy pupae, and any newly emerged adult *P. maculiventris* were removed, sexed, weighed, and individually transferred to plastic Petri dishes (80 × 15 mm) until use in the female longevity and reproduction experiment described below.

Female Longevity and Reproduction

Within a given treatment and block, *P. maculiventris* adults collected from the previous experiment were grouped into mating pairs. Each mating pair was then transferred to a single cabbage plant of the same treatment as when they were nymphs. The number of replicates, as well as the number of couples in each replicate varied with the adult emergence and sex ratio of each replicate from the previous experiment. Mealworms pupae were provided to *P. maculiventris* adults at a ratio of 2:1 or 3:1 per day. General maintenance of insects and plants were as described above. Adult survival was recorded every other day until the female death. For each evaluation date, eggs laid were transferred to a filter paper placed inside plastic Petri dishes (80 × 15 mm). Males were removed 15 d after the pairing (Torres and Zanuncio 2001, Torres et al. 2003). Petri dishes with eggs were maintained in the same growth chamber as the whole plant set-up. First-instar nymphs that hatched from eggs were counted and removed every other day. To avoid losing data from eggs that hatched belatedly, the plates were checked until 2 wk after the date of eggs were removed from plants. Data on number of eggs per female and the number of nymphs that hatched from these eggs were used to quantify female fecundity and fertility, respectively.

Statistical Analyses

Each experiment (nymph survival and development; female longevity and reproduction) was a randomized complete block design, with imidacloprid treatment as the main factor of interest. For a given treatment, there was one plant/replicate per block, and there were eight blocks across time, giving eight replicates per treatment total for each experiment. Survival data were subjected to survival analyses using Kaplan–Meier estimators (Log-Rank method), and insects surviving to adulthood were treated as censored data. Survival curves generated were compared using the Holm–Sidak method in SigmaPlot (Systat Software Inc. 2013). Data on developmental time (third instar to adult), median survival time (LT_{50}), adult emergence, sex rate, and body weight were subjected to regression analyses using the curve-fitting procedure of TableCurve 2D (Systat Software 2002), with insecticide concentration as the independent variable. Model selection was based on parsimony, high *F*-values (and mean square of errors), and steep increases in R^2 values. Data on female longevity, number of eggs laid, and number of nymphs hatched from these eggs were subjected to analyses of variance. Subsequent pairwise comparisons were made using Student's *t*-test ($P < 0.05$, PROC TTEST; SAS) (SAS Institute 2011). Residuals were checked for normality and homoscedasticity for all datasets and no data transformations were necessary.

Results

Nymph Survival and Development

Imidacloprid soil drenching of cabbage plants significantly reduced survival of *P. maculiventris* nymphs (log-rank test, $\chi^2 = 138.88$, df= 4; $P < 0.001$) following a concentration-dependent trend (Fig. 1a). Nymph median survival times (LT_{50}) ranged from 5 to 21 d depending on imidacloprid concentration (Fig. 1b). Despite differences in survival and median lethal time, the developmental time of surviving nymphs did not differ among treatments (20.93 ± 0.59 d; $F_{1, 24}$= 0.01; P = 0.93).

Adult Emergence, Body Weight, Longevity, and Fertility

Emergence of adult *P. maculiventris* decreased significantly with imidacloprid concentration applied as a soil drench (Fig. 2). The lowest adult emergence occurred with the recommended field rate of imidacloprid, where only one adult (female) emerged. The low number of individuals reaching the adult stage prevented assessment of adult longevity and fertility at higher imidacloprid concentrations. Thus, longevity and reproduction were compared for the control and the lowest imidacloprid concentration (0.504 mg imidacloprid $plant^{-1}$) only. Treatment did not significantly influence sex ratio (0.55 ± 0.05 female: 0.45 ± 0.05 male; $F_{1, 24}$ = 2.1, P = 0.16) or body weight, irrespective of sex (male = 42.51 ± 1.53 mg ($F_{1, 18}$ = 0.22, P = 0.65); female = 52.77 ± 1.34 mg ($F_{1, 20}$ = 0.35, P = 0.56). In contrast, the

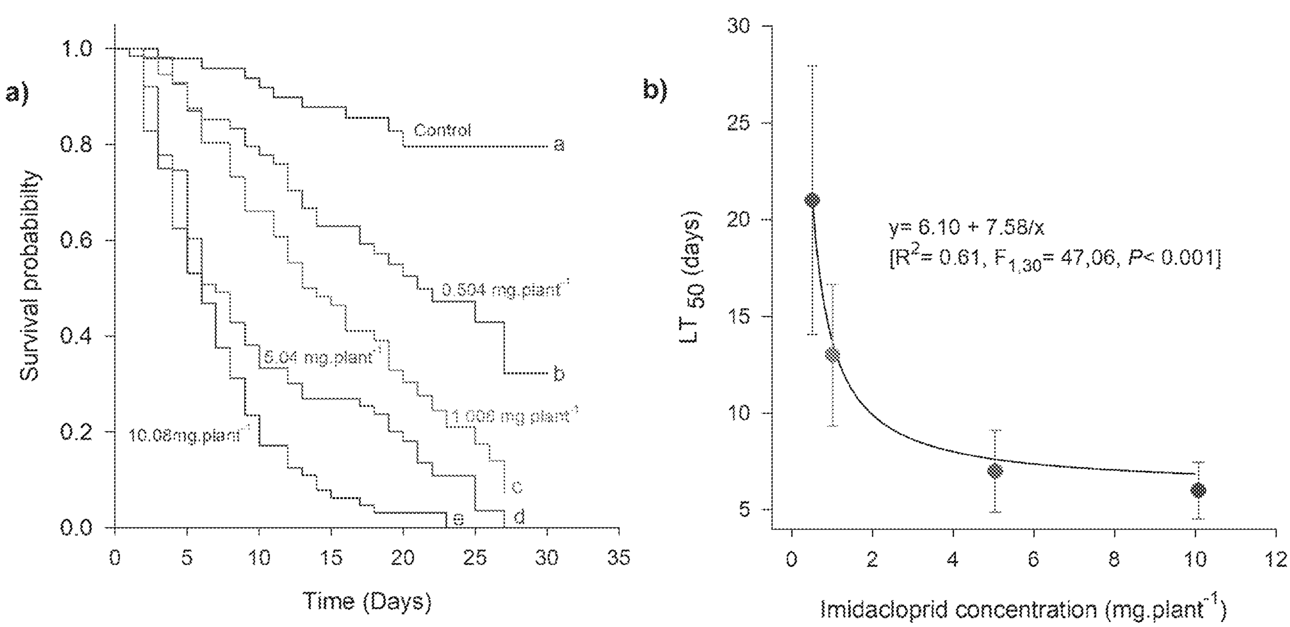

Fig. 1. Survival curves (a) and median lethal times (mean ± SE; LT_{50}) (b) of *P. maculiventris* nymphs reared in cabbage plants treated with imidacloprid by soil drenches. Survival curves with different lower-case letters are significantly different (Holm–Sidak test, $P < 0.05$).

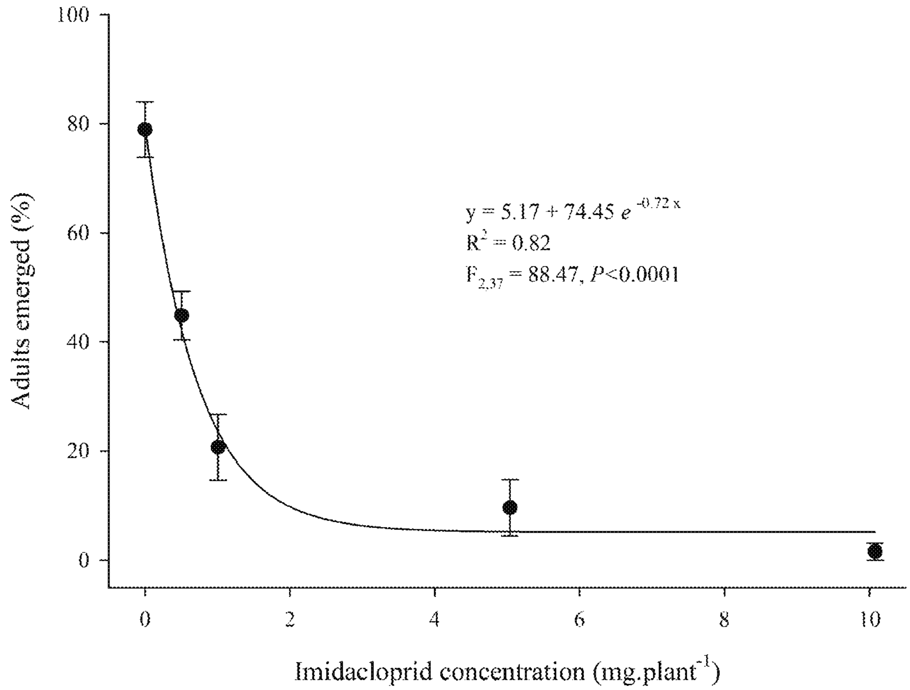

Fig. 2. Mean adult emergence (± SE) of *P. maculiventris* reared on cabbage plants subjected to imidacloprid soil drenching.

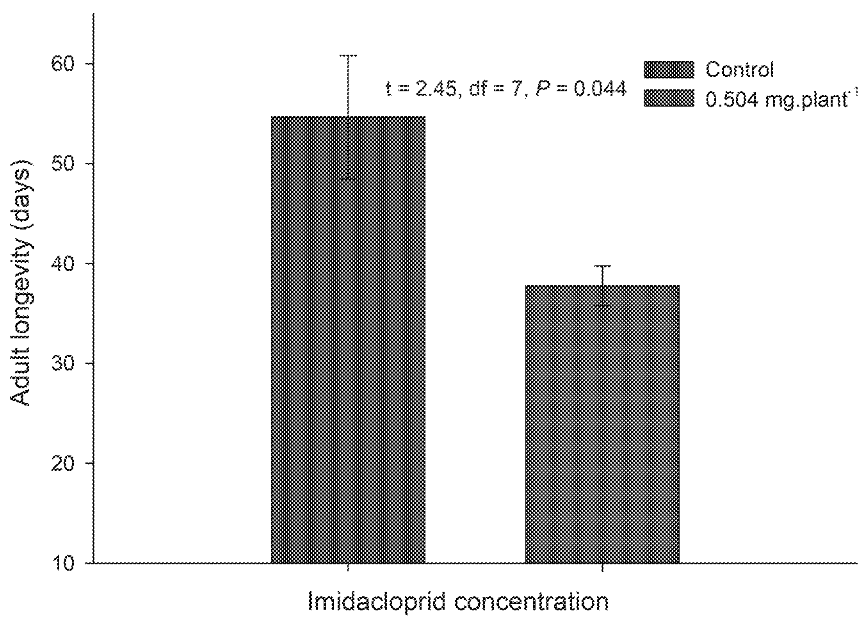

Fig. 3. Longevity (mean ± SE) of *P. maculiventris* females reared in cabbage plants subjected to imidacloprid soil drenching.

imidacloprid significantly reduced adult female longevity by almost 17 d (Fig. 3), fecundity (control: 448.13 ± 67.91; imidacloprid: 255.00 ± 28.45; Fig. 4a), and fertility (control: 374.82 ± 56.96; imidacloprid: 183.38 ± 21.61; Fig. 4b).

Discussion

We hypothesized that the application of imidacloprid soil drenches to cabbage would have negative effects on the spined soldier bug *P. maculiventris* maintained under continuous ingestion exposure. We suspected that this method of neonicotinoid application to prevent damage of early season pests in many crop systems, together with the zoophytophagous feeding habit of *P. maculiventris*, would subject the predator to toxic exposure levels via plant sap ingestion. This expectation was confirmed for most endpoints measured in this study.

Prolonged imidacloprid exposure of *P. maculiventris* via plant-feeding compromised nymph survival. This result is consistent with reported effects of high toxicity of imidacloprid to immatures of this species when exposed via contaminated water, treated foliage, or direct contact through topical spray (De Cock et al. 1996, Cutler et al. 2006). Nevertheless, our study indicates that when applied through soil drenching, the imidacloprid uptake by cabbage plants can also be lethal to *P. maculiventris* nymphs feeding on these plants.

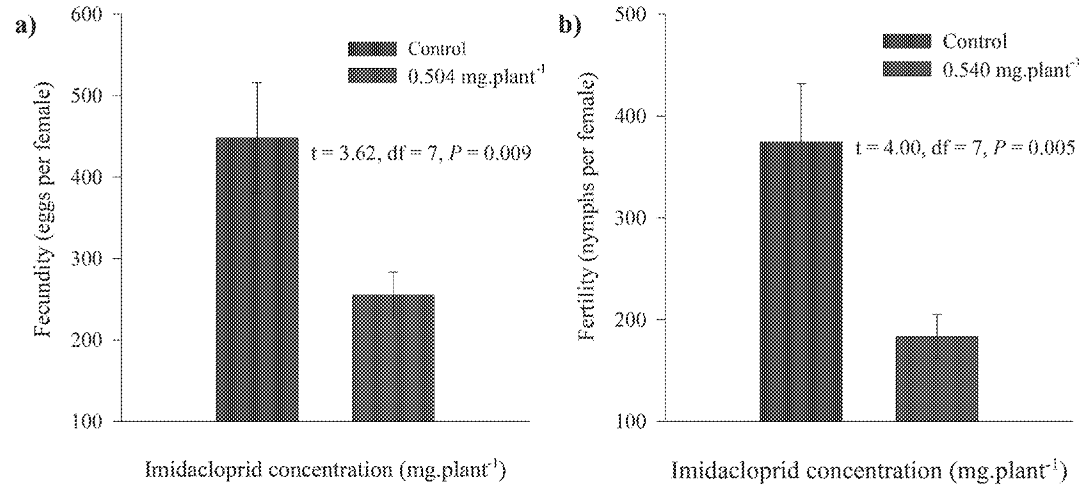

Fig. 4. Mean (± SE) fecundity (a) and fertility (b) of P. *maculiventris* females reared on cabbage plants subjected to imidacloprid soil drenching.

Other studies with predatory insects have similarly shown that significant lethal effects can occur with zoophytophagous predators that feed on plants systemically treated with neonicotinoids (Torres and Ruberson 2004; Rogers et al. 2007; Moser and Obrycki 2009; Gontijo et al. 2014, 2015, 2018; Bredeson and Lundgren 2018).

Negative effects of imidacloprid soil drenches on *P. maculiventris* occurred in a concentration-dependent manner. Studies of the persistence of imidacloprid in other crops show the insecticide concentration starts decreasing on plant tissues after around 10–15 d (Juraske et al. 2009, Sharma and Singh 2014). Changes over time in imidacloprid concentration in plant tissues were not measured in our study, but previous studies and our results indicate that lethal effects of imidacloprid soil drenching to the spined soldier bug probably subside over time. Systemically applied neonicotinoids may compromise life history traits of exposed zoophytophagous predators even under sublethal concentrations (Torres et al. 2003, Gontijo et al. 2015, Sâmia et al. 2018). Of insects that survived imidacloprid exposure in our experimental treatments, we did not notice any effect on predator developmental time or body weight. The absence of imidacloprid effects on such parameters may be a consequence of the lower number of individuals that reached the adult phase (which were used to record such parameters), especially on higher concentrations of the insecticide, and potentially uneven exposure. However, adult longevity, fecundity, and fertility were compromised by the plant-mediated imidacloprid exposure.

The primary mode of action of neonicotinoids is the competitive modulation as agonists of nicotinic acetylcholine receptors in neural synapses (Nauen and Bretschneider 2002, Tomizawa and Casida 2005, Jeschke et al. 2011, Casida and Durkin 2013). This action is irreversible under high insecticide concentrations, but lower concentrations of imidacloprid can lead to reversible sublethal effects such as antifeedant activity (Nauen 1995, Nauen and Elbert 1997, Boina et al. 2009). High median survival values of soldier bug nymphs feeding on plants treated with diluted imidacloprid concentrations could be a result of such antifeedant activity. However, lower body weights of newly emerged adults, an expected consequence of this effect, was not observed in our study, despite this effect being observed in another soldier bug species (Torres et al. 2003).

Females of *P. maculiventris* exposed to plants treated with imidacloprid soil drenches as low as one-twentieth the recommended label rate exhibited reduced longevity by about 17 d, and fecundity and fertility were less than half that of females from untreated plants. These results contrast with those of De Cock et al. (1996), who found sublethal ingestion of imidacloprid did not affect the longevity or fertility of *P. maculiventris*. Both negative and positive effects of imidacloprid on such parameters have been reported for other natural enemies (Xiao et al. 2016, Fernanda et al. 2017, D'Ávila et al. 2018), and piercing-sucking pests (Qu et al. 2015, Haddi et al. 2016, Rix et al. 2016, Santos et al. 2016). However, all these studies assessed residual contact exposure, not sap-feeding of plants contaminated by uptake of soil-drenching insecticide.

In summary, our results suggest that feeding upon imidacloprid soil-drenched cabbage plants would be harmful to the zoophytophagous biocontrol agent *P. maculiventris*, with potential ramifications on nymph survival, adult emergence and longevity, and reproduction. Such deleterious effects on biocontrol agents should be considered when using imidacloprid soil drenches in pest management programs.

Acknowledgments

Financial support was provided by the Minas Gerais State Foundation for Research Aid (FAPEMIG BDS-00488-16), National Council of Scientific and Technological Development (CNPq), CAPES Foundation (Brazilian Ministry of Education; Finance Code 001), and a Discovery Grant from the Natural Sciences and Engineering Research Council of Canada (RGPIN-2015–04639).

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